

**CLAIMS**

1. A method of identifying a uniquely targeting siRNA nucleotide sequence for a target mRNA sequence of a target species comprising the steps of:

comparing a database of mRNA sequences from the target species with an siRNA nucleotide sequence that consists of 18-25 including at least 11 consecutive nucleotides complementary to the target mRNA sequence to be cleaved by the siRNA nucleotides, wherein the at least 11 consecutive nucleotides complementary to the target mRNA sequence include a nucleotide that is third from an siRNA nucleotide sequence's 5' end; and

determining if, in addition to the target mRNA sequence, one or more additional mRNA sequences in the database are complementary to an 11 consecutive nucleotide sequence of the siRNA nucleotide sequence including the third nucleotide from the 5' end of the siRNA nucleotide,

wherein an absence of one or more additional mRNA sequences in the database that are complementary to an 11 consecutive nucleotide sequence of the siRNA nucleotide sequence including the third nucleotide from the 5' end of the siRNA nucleotide indicates that the siRNA nucleotide sequence is a uniquely targeting siRNA nucleotide sequence.

2. A method of designing a uniquely targeting siRNA for a target mRNA molecule comprising the steps of:

a) identifying an siRNA nucleotide sequence for the target mRNA, said sequence consisting of 18-24 nucleotides including a nucleotide sequence that has 11 consecutive nucleotides, including the third nucleotide from the siRNA nucleotide sequence's 5' end, that are complementary to an 11 nucleotide sequence that occurs on the target mRNA molecule;

comparing the siRNA nucleotide sequence with a database of mRNA sequences from the target mRNA species; and

determining if, in addition to the target mRNA sequence, one or more additional mRNA sequences in the database are complementary to an 11 consecutive nucleotide sequence of the siRNA nucleotide sequence including the third nucleotide from the 5' end of the siRNA nucleotide,

wherein an absence of one or more additional mRNA sequences in the database that are complementary to an 11 consecutive nucleotide sequence of the siRNA nucleotide sequence including the third nucleotide from the 5' end of the siRNA nucleotide indicates that the of the siRNA nucleotide is a uniquely targeting siRNA nucleotide sequence.

3. The method of claim 1 or 2 wherein the database of mRNA sequences is a selected from the group consisting of: NCBI database and ENSEMBL database.
4. The method of any of claims 1 to 3 wherein the comparing of the siRNA nucleotide sequence with the database of mRNA sequences from the target mRNA species is performed by a computer.
5. The method of any of claims 1 to 4 wherein the comparing of the siRNA nucleotide sequence with the database of mRNA sequences from the target mRNA species is performed by a computer using a BLAST program.
6. The method of any of claims 1 to 5 wherein the siRNA nucleotide sequence consists of 18, 19, 20 or 21 nucleotides.
7. The method of any of claims 1 to 5 wherein the siRNA nucleotide sequence consists of 22, 23, 24 or 25 nucleotides.
8. The method of any of claims 1 to 7 wherein the target mRNA is from an animal.
9. The method of any of claims 1 to 8 wherein the target mRNA is from an animal selected from the group consisting of: a human, a canine species, a feline species, an equine species, a bovine species, a porcine species, an ovine species, an avian species and fish.
10. The method of any of claims 1 to 9 wherein the target mRNA is selected from the group consisting of: an mRNA encoding an oncogene, an mRNA encoding a

pathogen protein, a cytokine, a chemokine, a co-stimulatory molecule and a growth factor.

11. The method of any of claims 1 to 10 wherein the target mRNA is selected from the group consisting of: an mRNA encoding a viral protein and an mRNA encoding a bacterial protein.

12. The method of any of claims 1 to 11 wherein the target mRNA is selected from the group consisting of: an mRNA encoding VEGF, an mRNA encoding an oncogene product of *bcr/abl*, an mRNA encoding an HIV protein, an mRNA encoding an HSV protein, an mRNA encoding an HCV protein and an mRNA encoding a CMV protein.

13. A method of synthesizing a uniquely targeting siRNA for a target mRNA molecule comprising the steps of:

identifying or designing uniquely targeting siRNA nucleotide sequence for the target mRNA according to any of claims 1 to 12; and

synthesizing an siRNA molecule having the uniquely targeting siRNA nucleotide sequence.

14. A method of inhibiting expression of a target mRNA molecule comprising the steps of:

synthesizing a uniquely targeting siRNA for a target mRNA molecule according to claim 13; and

contacting a target mRNA molecule with an RISC that comprises an siRNA molecule that comprises the uniquely targeting siRNA nucleotide sequence.

15. A method of identifying an miRNA nucleotide sequence that does not function as a siRNA nucleotide sequence for mRNA of a target species comprising the steps of:

comparing a database of mRNA sequences from the target species with an miRNA nucleotide sequence that consists of 18-24 nucleotides; and

determining if one or more mRNA sequences in the database are complementary to an 11 consecutive nucleotide sequence of the miRNA nucleotide sequence including the third nucleotide from the 5' end of the miRNA nucleotide, wherein an absence of one or more mRNA sequences in the database that are complementary to an 11 consecutive nucleotide sequence of the miRNA nucleotide sequence including the third nucleotide from the 5' end of the miRNA nucleotide indicates that the miRNA nucleotide sequence does not function as a siRNA nucleotide sequence for mRNA of a target species.

16. A method of designing an miRNA nucleotide sequence that does not function as a siRNA nucleotide sequence for mRNA of a target species comprising the steps of:

identifying an miRNA nucleotide sequence that consists of 18-24 nucleotides;  
comparing the miRNA nucleotide sequence with a database of mRNA sequences from the target mRNA species; and  
determining if one or more mRNA sequences in the database are complementary to an 11 consecutive nucleotide sequence of the miRNA nucleotide sequence including the third nucleotide from the 5' end of the miRNA nucleotide, wherein an absence of one or more mRNA sequences in the database that are complementary to an 11 consecutive nucleotide sequence of the miRNA nucleotide sequence including the third nucleotide from the 5' end of the siRNA nucleotide indicates that the miRNA nucleotide sequence does not function as a siRNA nucleotide sequence for mRNA of a target species.

17. The method of claim 15 or 16 wherein the database of mRNA sequences is selected from the group consisting of: NCBI database and ENSEMBL database.

18. The method of any of claims 15 to 17 wherein the comparing of the miRNA nucleotide sequence with the database of mRNA sequences from the target species is performed by a computer.

19. The method of any of claims 15 to 18 wherein the comparing of the miRNA nucleotide sequence with the database of mRNA sequences from the target species is performed by a computer using a BLAST program.
20. The method of any of claims 15 to 19 wherein the miRNA nucleotide sequence consists of 18, 19, 20 or 21 nucleotides.
21. The method of any of claims 15 to 19 wherein the miRNA nucleotide sequence consists of 22, 23, 24 or 25 nucleotides.
22. The method of any of claims 15 to 21 wherein the target species is an animal.
23. The method of any of claims 15 to 22 wherein the target species is an animal selected from the group consisting of: a human, a canine species, a feline species, an equine species, a bovine species, a porcine species, an ovine species, an avian species and fish.
24. The method of any of claims 15 to 23 wherein the miRNA inhibits translation of mRNA selected from the group consisting of: an mRNA encoding an oncogene, an mRNA encoding a pathogen protein, a cytokine, a chemokine, a co-stimulatory molecule and a growth factor.
25. The method of any of claims 15 to 24 wherein the miRNA inhibits translation of mRNA selected from the group consisting of: an mRNA encoding a viral protein and an mRNA encoding a bacterial protein.
26. The method of any of claims 15 to 25 wherein the target mRNA is selected from the group consisting of: an mRNA encoding VEGF, an mRNA encoding an oncogene product of *bcr/abl*, an mRNA encoding an HIV protein, an mRNA encoding an HSV protein, an mRNA encoding an HCV protein and an mRNA encoding a CMV protein.
27. A method of synthesizing an miRNA that does not function as a siRNA nucleotide sequence for mRNA of a target species comprising the steps of:

identifying or designing an miRNA nucleotide sequence that does not function as an siRNA nucleotide sequence for mRNA of a target species according to any of claims 15 to 26; and

synthesizing an miRNA molecule that has an miRNA nucleotide sequence that does not function as an siRNA nucleotide sequence for mRNA of a target species

28. A method of inhibiting expression of a target mRNA molecule comprising the steps of:

synthesizing an miRNA that does not function as a siRNA nucleotide sequence for mRNA of a target species according to claim 27; and

contacting a target mRNA molecule with an miRNP that comprises an miRNA that does not function as a siRNA nucleotide sequence for mRNA of a target species.

29. A uniquely targeting siRNA molecule consisting of 18-25 nucleotides including a nucleotide sequence that has at least 11 consecutive nucleotides, including the third nucleotide from the 5' end of the siRNA molecule, that are complementary to a nucleotide sequence that occurs on a target mRNA molecule and not on another mRNA from a target species.

30. The uniquely targeting siRNA molecule of claim 29 wherein the nucleotide sequence of the siRNA molecule is fully complementary with a sequence of the target mRNA molecule.

31. An siRNA molecule consisting of 18-25 nucleotides including a nucleotide sequence that has at least 11 consecutive nucleotides, including the third nucleotide from the 5' end of the siRNA molecule, that are complementary to a nucleotide sequence that occurs on a target mRNA molecule and not on another mRNA from a target species, wherein the siRNA molecule is not fully complementary with a sequence of the target mRNA molecule.

32. The siRNA molecule of claim 31 consisting of 18, 19, 20 or 21 nucleotides.

33. The siRNA molecule of claim 31 consisting of 22, 23, 24 or 25 nucleotides.
34. The siRNA molecule of any of claims 29 to 33 wherein the target mRNA is from an animal.
35. The siRNA molecule of any of claims 29 to 34 wherein the target mRNA is from an animal selected from the group consisting of: a human, a canine species, a feline species, an equine species, a bovine species, a porcine species, an ovine species, an avian species and fish.
36. The siRNA molecule of any of claims 29 to 35 wherein the target mRNA is selected from the group consisting of an mRNA encoding: an oncoprotein, a tumor suppressor, a pathogen protein, a cytokine, an interleukin, a chemokine, a co-stimulatory molecule and a growth factor.
37. The siRNA molecule of any of claims 29 to 36 wherein the target mRNA is selected from the group consisting of: an mRNA encoding a viral protein and an mRNA encoding a bacterial protein.
38. The siRNA molecule of any of claims 29 to 37 wherein the target mRNA is selected from the group consisting of: an mRNA encoding p53, MYC, MYB, PTEN, HER-2/NEU, TGF, PDGFR, Ras, Rb (Retinoblastoma protein), bcl-2, bax, phosphatase 2A, telomerase, p16, p21, Cyclin Dependent Kinases (CDKs), TRAIL, TNF, Mitogen Activated Kinases (MAPKs), VHL, NfκB, Merlin, Schwanomin, Notch, APC, AXIN2, E-Cadherin, GPC3, AXT1,2, Patched, SUFU, FH, SDHB,C,D, WT-1, STK11, TSC1, TSC2, BMPR1A, SMAD4, NF2, BHD, HRPT2, MUTYH, ATM, BLM, BRCA1, BRCA2, FANCA,C,D2,E,F,G, MSH2, MLH1, MSH6, PMS2, XPA, KT, MET, RET, HIF-1, b-catenin, FAS, FBXW7, GLI, HPEVE6, MDM2, AKT2, FOXO1A,3A, PI3KCA, CYCLIN D1, HPVE7, TAL1, TFE3, ALK, ABL1, BRAF, EGFR, EPHB2, ERBB2, FES, FGFRs, FLT3,4, JAK2, N-RAS, K-RAS, PDGFB, PDGFRB, EWSR1, RUNX1, SMAD2, TGFFBR1, 2, MYCN, HMGA2, HOXA9,11,13, HOX11,11L2, MKK4, MLL, SS18, RARA, PTNP1,11, APP (Amyloid Precursor protein), Tau, synucleins, alpha-synuclein (PARK1), Parkin (PARK2), PARK5, Huntingtin, SOD (Superoxide Dismutase), Senataxin,

Apolipoprotein-E, DJ-1 (PARL7), presenilin-1, presenilin-2, IL-1, IL-2, IL-4, TNF, Interferons, (IFNA, IFNB, IFN-gamma), CD4, CD8, CD19, CD5, CD43, CD1, CD3, B-cell receptor, T-cell Receptor, VEGF, *bcr/abl*, HIV genes such as *rev*, *env*, *gag*, or CMV genes.

39. A RISC comprising an siRNA molecule of any of claims 29 to 37.

40. An miRNA molecule for a target mRNA of a target species, wherein said miRNA molecule consists of 18-25 nucleotides and does not function as a siRNA nucleotide sequence for mRNA of a target species.

41. The miRNA molecule of claim 40 consisting of 18, 19, 20 or 21 nucleotides.

42. The miRNA molecule of claim 40 consisting of 22, 23, 24 or 25 nucleotides.

43. The miRNA molecule of any of claims 40 to 42 wherein the target species is an animal.

44. The miRNA molecule of any of claims 40 to 43 wherein the target species is from an animal selected from the group consisting of: a human, a canine species, a feline species, an equine species, a bovine species, a porcine species, an ovine species, an avian species and fish.

45. The miRNA molecule of any of claims 40 to 44 wherein the target mRNA is selected from the group consisting of an mRNA encoding: an oncoprotein, a tumor suppressor, a pathogen protein, a cytokine, an interleukin, a chemokine, a co-stimulatory molecule and a growth factor.

46. The miRNA molecule of any of claims 40 to 45 wherein the target mRNA is selected from the group consisting of: an mRNA encoding a viral protein and an mRNA encoding a bacterial protein.

47. The miRNA molecule of any of claims 40 to 46 wherein the target mRNA is selected from the group consisting of: an mRNA encoding p53, MYC, MYB, PTEN,



HER-2/NEU, TGF, PDGFR, Ras, Rb (Retinoblastoma protein), bcl-2, bax, phosphatase 2A, telomerase, p16, p21, Cyclin Dependent Kinases (CDKs), TRAIL, TNF, Mitogen Activated Kinases (MAPKs), VHL, NfκB, Merlin, Schwannomin, Notch, APC, AXIN2, E-Cadherin, GPC3, AXT1,2. Patched, SUFU, FH, SDHB,C,D, WT-1, STK11, TSC1, TSC2, BMPR1A, SMAD4, NF2, BHD, HRPT2, MUTYH, ATM, BLM, BRCA1, BRCA2, FANCA,C,D2,E,F,G, MSH2, MLH1, MSH6, PMS2, XPA, KT, MET, RET, HIF-1, b-catenin, FAS, FBXW7, GLI, HPEVE6, MDM2, AKT2, FOXO1A,3A, PI3KCA, CYCLIN D1, HPVE7, TAL1, TFE3, ALK, ABL1, BRAF, EGFR, EPHB2, ERBB2, FES, FGFRs, FLT3,4, JAK2, N-RAS, K-RAS, PDGFB, PDGFRB, EWSR1, RUNX1, SMAD2, TGFFBR1, 2, MYCN, HMGA2, HOXA9,11,13, HOX11,11L2, MKK4, MLL, SS18, RARA, PTNP1,11, APP (Amyloid Precursor protein), Tau, synucleins, alpha-synuclein (PARK1), Parkin (PARK2), PARK5, Huntingtin, SOD (Superoxide Dismutase), Senataxin, Apolipoprotein-E, DJ-1 (PARL7), presenilin-1, presenilin-2, IL-1, IL-2, IL-4. TNF, Interferons, (IFNA, IFNB, IFN-gamma), CD4, CD8, CD19, CD5, CD43, CD1, CD3, B-cell receptor, T-cell Receptor, VEGF, *bcr/abl*, HIV genes such as *rev*, *env*, *gag*, or CMV genes.